ESTIMATION OF THE PREVALENCE OF LATENT TUBERCULOSIS INFECTION AMONG HOUSEHOLD CONTACTS OF SPUTUM CULTURE POSITIVE TUBERCULOSIS PATIENTS IN VELLORE, SOUTH INDIA

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1. INTRODUCTION

Tuberculosis still remains a major public health problem despite the fact that the causative organism was discovered some 100 years back and highly effective drugs are available for its treatment.

Worldwide 9.1 million cases are detected annually out of which half are sputum positive .[1] South East Asia which account for 25% of the world's population accounts for 34% of world's tuberculosis burden .[1]

WHO has identified 22 high burden countries in terms of tuberculosis incidence which account for 80% of global tuberculosis burden.[1]

India ranks 17th among 22 high burden countries in terms of tuberculosis incidence rate.[1] India is the country with the highest burden of tuberculosis in the world accounting for 1/8th of the global incidence of tuberculosis.[2] Annually, around 1.9 million cases of tuberculosis are detected in India out of which 0.8 million are sputum positive infectious cases.[2]

In India, everyday more than 5000 people develop tuberculosis disease and nearly 1000 die due to tuberculosis i.e. 2 deaths every 3 minutes.[2] Deaths due to tuberculosis exceed the combined deaths due to all other communicable diseases and account for 26% of all avoidable deaths in adults.[2]

Tuberculosis affects the most productive age group. Of all tuberculosis cases in India 2/3rd are male and 70% of all patients are aged between 15 and 54 years.[3] More than 3,00,000 children are forced to leave school because their parents have tuberculosis and about 1,00,000 women with tuberculosis are rejected by their families annually.[3]

Direct and indirect cost of tuberculosis to India for morbidity alone amounts to an estimated $ 3 billion annually (2000).[3] Studies suggested on an average 3 to 4 months of work time is lost as a result of tuberculosis, resulting in an average potential loss of 20-30% of the annual household income.[3]

There is an increasing concern about drug resistant tuberculosis. Several small surveys conducted across the country have shown the prevalence rates of Multi Drug Resistant Tuberculosis (strains of Mycobacterium tuberculosis resistant to both Isoniazid and Rifampicin) of around 3% among new cases and 12% among retreatment cases.[4, 5]

With increase in incidence of HIV infection , there is increasing focus on HIV-TB co-infection. An HIV infected person co-infected with tubercle bacilli has 50-60% life time risk of developing tuberculosis disease as compared to a 10% life time risk of developing tuberculosis disease in a non-HIV infected person.[6] Tuberculosis infection refers to a positive tuberculin skin test with or without signs and symptoms of disease. Tuberculosis disease refers to cases who have positive acid-fast smear culture from Mycobacterium tuberculosis or radiographic and clinical presentation of tuberculosis. Investigation of people exposed to cases of infectious tuberculosis (contact investigation) is the key to tuberculosis control in countries with low tuberculosis incidence. However, in countries in which the incidence of tuberculosis is high (like India); contact investigation is not commonly done.

2. JUSTIFICATION

The status of the disease and disease trends is one of the basic requirements in the development of control programme for that disease and its subsequent evaluation. Subjects in contact with infective pulmonary tuberculosis may be infected if the index case coughs and expels Mycobacteria into the air. Infected contacts will then develop a progressive immune response and some (between 5-10% of those who are exposed) will develop tuberculosis disease within their lifespan. This progression from infection to disease will depend on several factors , such as age, sex, size of tuberculin reaction, immune status, diabetes, smoking, drug treatment and nutritional status.[7,8,9,10] The risk of developing tuberculosis disease from tuberculosis infection is maximum in the first 2 years following infection and decreases with time.[11]

In all countries the first priority is rapid detection of cases and their prompt treatment. In regions with a high incidence of tuberculosis a search for contacts among the relatives of smear positive cases may succeed in detecting large number of secondary cases, but the search for infected contacts is less of a priority except among close relatives and small children, who may rapidly develop severe forms of the disease.[12]

Soon after the introduction of antibiotics (as early as 1959), trials were conducted which demonstrated that, if properly prescribed and taken, preventive treatment reduces the risk of future disease and is cost-effective.[13, 14]

In theory at least, if applied rigorously to infected individuals or to whole population with a high rate of latent tuberculosis infection, this policy could be successful in lowering the incidence of tuberculosis in future.[16]

As individuals with latent infection with tuberculosis by definition are healthy and do not have radiological abnormalities (except in few cases), screening must rely on immunological markers of

infection. For nearly a century the screening for latent tuberculosis infection relied on Tuberculin Skin Testing (TST). While TST is a useful guide for identifying tuberculosis infection, it has a number of drawbacks including the need for a repeat visit to read the test, problems in interpretation due to cross-reactivity with other mycobacterial species, the booster effect, and false negative results because of intercurrent immunosupression, as well as the variability inherent in its application and reading.[17]The recent introduction of T-cell based interferon gamma assay (IFN-γ) has demonstrated a role in screening for Mycobacterium tuberculosis infection, contact tracing, and has reportedly overcome some of the drawbacks of TST.[18,19,20,21]

Most of these studies demonstrating the efficacy of interferon gamma assay are in low incidence countries. More studies are needed for finding the prevalence of latent tuberculosis infection in high prevalence countries using TST and the new IGRA test.

The present study will serve two purposes. Firstly, it will help in contributing to the present knowledge about the prevalence of latent tuberculosis infection among household contacts of infective tuberculosis cases. Secondly, this study will also measure the agreement between TST and IGRA tests in diagnosing latent tuberculosis infection in household contacts of infective tuberculosis cases.

3. OBJECTIVES

1. To estimate the prevalence of latent tuberculosis infection in the household contacts of sputum culture positive tuberculosis patients among suspected multi drug resistant tuberculosis patients in a population covered by CHAD Tuberculosis Unit and District Tuberculosis Centre TB Unit in Vellore district using tuberculin skin testing (TST) and whole blood interferon gamma (i.e. Quantiferon TB –Gold).

2. To find the agreement between Quantiferon TB-Gold and TST in detecting latent tuberculosis infection in the above population.

4. LITERATURE REVIEW

4.1 Magnitude of the Problem

Tuberculosis represents a serious public health issue worldwide. The 2008 World Health Organization Report on tuberculosis states that a total of 9.2 million new cases (139 per 1,00,000 population) and 1.7 million deaths from tuberculosis occurred worldwide in 2006.[1] Deaths due to tuberculosis comprises 25% of all avoidable deaths in developing countries.[1] In developing countries 75% of all tuberculosis cases die in the productive age group of 15 – 50 years. [1]

The largest number of cases occur in South East Asia which accounts for 34% of all incident cases globally.[1] In 2007 there were an estimated 5.7 million cases in this region. Every year, 3 million people develop active tuberculosis in this region and 50,000 die.[1] In South East Asia 80% of all cases are in the age group of 15-54 years.[1] Bangladesh, India, Indonesia, Myanmar and Thailand accounted for 95% of global tuberculosis burden.[1]

Tuberculosis is the biggest public health problem in India. With 1.8 million new cases occurring annually, India accounts for a fifth of the world's new cases and 2/3rd of all the new cases in South East Asia.[2] More than 80% of the burden of tuberculosis is due to premature deaths, as measured in terms of Disability Adjusted Life Years (DALY) lost.[2]Among all cases 70% occur in the economically productive age group of 15-54 years.[2] Tuberculosis causes huge economic loss with about 17 crores workdays lost due to the disease every year.[3]

It is estimated that between 2002 and 2020, approximately 1 billion people will be newly infected, over 150 million will get sick and 36 million will die of tuberculosis, if control measures are not strengthened.[3]

4.2 Diagnosing Latent Tuberculosis Infection

Latent tuberculosis infection is a sub clinical infection with Mycobacterium tuberculosis without clinical, bacteriological, or radiological evidence of tuberculosis disease. The standard test for

diagnosis of latent tuberculosis infection is TST.

4.2.1 Standard Tuberculin Test

4.2.1.1 Tuberculin

The tuberculin skin test has been the standard method of diagnosing infection with Mycobacterium tuberculosis. Tuberculin skin testing is done using a purified protein derivative (PPD) whose strength is usually expressed in terms of Tuberculin Unit. The commercially available PPD preparation is defined as the dose of that product which is biologically equivalent to that contained in 5 Tuberculin unit of PPD-S (PPD –Seibert) i.e., it elicits skin reaction of same size as PPD-S with a error of 20%.[24] One Tuberculin

Unit of Purified Protein Derivative RT 23 with Tween 80 corresponds fairly well to 3 TU of PPD-S. Therefore, 2TU of PPD RT 23 with Tween 80 is used for diagnosis or survey.[26] Tween 80 is a stabilizing agent to prevent the absorption of tuberculin to glass surfaces. Purified protein derivative (PPD) RT 23 with Tween 80 is prepared from Mycobacterium tuberculosis by Statens Serum Institute, Copenhagen in Denmark and the seed lot is supplied in freeze dried form to laboratories of individual countries. Other tuberculin available in the market may not be standardized.

Tuberculin vials should be always stored at 2°C - 8°C and used before the expiry period, which is about one year after reconstitution and dilution. Exposure to sunlight and heat must be avoided.[27] The tuberculin should not be allowed to freeze or kept at temperatures above 20°C except for very short period.

4.2.1.2 Administration of the Test

Tuberculin is injected in a measured amount of 0.1 ml intradermally on the mid-volar aspect of the forearm (Mantoux Method). Conventionally the test is given on the left forearm to avoid errors in reading. However, right arm can be used in case of any contraindication to use the left arm.

The skin is lightly stretched and the needle point is inserted with the bevel pointing upwards

into the superficial layers of the skin. The area chosen should be free of scars, veins and any inflammation. The test site need not be sterilized before injection.[28] It can be simply washed with soap and water and dried before injection. The injection is given with the standard 1 ml tuberculin syringe graduated to hundredth of a millimeter fitted with 26-gauge needle of half an inch length and 20° bevel. A glass tuberculin syringe or a disposable tuberculin syringe can be used. No other syringe like a insulin syringe should be used for this purpose.

A satisfactory test should raise a flat pea-sized wheel of diameter 6mm to 10mm with clear pits of hair follicles. There should be no leaking of tuberculin. If the test is unsatisfactory i.e., the correct amount has not been injected or the injection has been made into the subcutaneous tissue , then another injection can be given either at a sufficient distance from the first injection site or in the other forearm.

In some atopic individuals, an urticarial wheel may appear within minutes of injection. It usually disappears within 1 to 2 hours. The formation of vesicle, bullae, lymphangitis, ulceration or necrosis at the site, which may occur in a proportion of children, indicates a high degree of tuberculin sensitivity.[29]

4.2.1.3 Reading of the Test

The injection of the tuberculin antigen leads to migration and proliferation of sensitized T-cell lymphocytes to the test site. These T-cells release lymphokines, which further attracts other lymphocytes and monocytes.[30] This reaction along with the increased permeability of the local blood capillaries leads to an induration at the test site. The size of this induration is maximum between 48-96 hours after the test .[31] The reading of the test is done by measuring the transverse diameter of this induration during this period.[31] The reading of the test should be done in good day light with flexed forearm, by carefully palpating the site of injection using one finger. The test result should never be recorded as “positive” or “negative” and must always be recorded in millimeter of size. Record should also be of formation of vesicles, bullae, lympangitis, ulceration and necrosis at the test site.

4.2.1.4 Interpretation of Tuberculin Test

Population surveys have shown that there are two groups of individuals in any community, one consisting of those “infected with M.tuberculosis bacilli”, the rest have no tuberculin sensitivity or sensitivity due to some other causes. The majority of the reactions above a particular cut off point obtained from tuberculin surveys in respective areas signify infection with tuberculosis bacilli and the majority of the reactions below the cut off are due to other causes. However there is some degree of overlapping between the infected group and the rest even around the cut off points. Therefore at any given cut off point some true infections will be missed and some falsely included. These cut off points as obtained during epidemiological surveys have been found to vary between 10mm to 15mm in different parts of the country.[32] However it is impractical to conduct

tuberculin surveys all over the country to find suitable cut off points in respective areas. Therefore, the interpretation of reactions in 10mm- 14mm range requires more careful interpretation.[32]

Summary of Interpretation of the Tuberculin Test[30]

Size of Indurations 15mm or above

- Signifies infection with tubercle bacilli irrespective of BCG vaccination status

Size of Indurations between 10mm- 14mm

- Could be attributable to one of the following causes
    a. Cross reactivity induced by environmental mycobacteria
    b. BCG vaccination induced sensitivity
    c. Infection with tubercle bacilli

  It is more likely to be attributable to infection with tubercle bacilli in case of history of contact with infective cases of pulmonary tuberculosis, clinically confirmed TB, or X-ray findings suggestive of tuberculosis.

Size of Indurations between 5mm- 9mm

- Majority of such reactions are attributable to cross-sensitivity induced by environmental mycobacteria and/ or vaccination with BCG
- It could also be attributable to infection with tubercle bacilli in the presence of immunosuppression

Size of Indurations less than 5mm

- Indicates absence of any type of mycobacterial infections except in children with severe type of immune-suppression

4.2.1.5 Causes of False Negative Reactions to TST [33]

a. Infections (viral infections like measles, chicken pox and HCV, bacterial like recent or overwhelming tubercular infection, leprosy,brucellosis,associated fungal infections).
b. Faulty technique
c. Improper storage and dilution of tuberculin
d. Desensitization due to load of antigens as in military tuberculosis and tubercular meningitis
e. Attenuated vaccination against viral infections like measles,mumps,polio
f. Metabolic derangements
g. Chronic renal failure
h. Protein energy malnutrition
i. Lymphoreticular disorders
j. Lack of available circulating T-lymphocytes
k. Presence of abnormally high number of circulating T-suppressor cells at the site of skin test
l. Intake of corticosteroids, immunosuppressive drugs

m. Extremes of ages (newborn or elderly)

n. Stress (surgery, burns, grafts vs. host disorders

o. Denatured or contaminated tuberculin

p. Errors in reading / recording

4.2.1.6 Causes of False Positive Test

4.2.1.6.1 Infection with Non-Tuberculous Mycobacteria

Infection with environmental mycobacteria also causes sensitization of the host. The sensitivity induced by these environmental mycobacteria that are generally non-pathogenic, cross reacts with tuberculin and is known as non-specific sensitivity (NSS). This non-specific sensitivity is highly prevalent in most parts of India as in other non tropical countries. During the tuberculosis prevention trial at Chingelpet, India, 61% of children were found to be infected with environmental mycobacteria by the age of 9 years and almost all by 19 years.[34] Therefore, much of tuberculin sensitivity in the community is due to frequent contact with ubiquitous environmental mycobacteria. However, sensitivity induced by these mycobacteria will lead to smaller reactions to tuberculin than from infection with tubercle bacilli.[35]

4.2.1.6.2 Effect of Vaccination with BCG

The effect of vaccination with BCG on subsequent tuberculin skin test is highly variable as it partly depends on the strain of BCG used. In a study conducted by National Tuberculosis Institute in Bangalore (India), 70% of the children aged 0-9 years,

vaccinated under Universal Immunisation Programme, elicited either no reaction or a reaction less than 10mm.[36] Some studies have shown that the effect of vaccination with BCG on tuberculin skin test actually wanes over time.[37, 38] Seth et al have demonstrated that, there is 50-60% waning in the first year itself.[37] Wang et al, in a meta-analysis , showed that vaccination with BCG significantly increases the

likelihood of a positive tuberculin skin test. [38] They also showed that the effect of vaccination with BCG on TST was less after duration of 15 years and the reactions more than 15mm are more likely to be the result of tuberculosis infections rather than the BCG vaccination.[38]

4.2.1.6.3 Booster Phenomenon

On sequential tuberculin testing some persons show a marked increase in the size of their skin reactions that may not be due to recent or past infections. This is called “booster” phenomenon. The increase seems to occur as the initial test stimulates the factors that determines reaction size in the subsequent test.[39]

4.2.2 Other Tuberculin Test

4.2.2.1 Scariform Test[40]

There are two types of scariform tests – Von Pirquet Test and Trumbusti Test.

Von Pirquet Test

This test was first used by Clemens von Pirquet in 1907 for detecting allergy to tuberculin. It as described as the first safe method of detecting allergy to tuberculin. Two drops of 25% dilution of Old Tuberculin (OT) and two drops of control diluents was placed side by side over the test site in one forearm. Superficial scratches are made with a sharp knife or lancet of equal length and depth, through the diluents over the skin of the forearm. The tests are read after two days. A swollen linear scar of 3-4mm width through the OT indicates a positive reaction. The limitation of this test is that it does not measure allergy quantitatively.

Trumbusti Test

This test is a slight modification of the Von pirquet test. A single puncture is made through the concentrated drop of OT with a wide bore needle with a beveled edge and needle is turned two to three times so that a small puncture is cut by it. Tuberculin is allowed to dry on the spot. The test is read 2-3 days later.

4.2.2.2 Moro Patch Test[40]

Around 1908 Moro introduced the percutaneous patch test. Old Tuberculin jelly is placed on the skin of the arm or back and is covered with a plaster. A control test with only the jelly is placed next to the test. Both plasters are removed after a day and both tests are read 2-5 days later. Volmer in 1937 still modified the patch test in which the Old

Tuberculin was dried on adhesive tape and was stuck to the skin. A control patch was applied. The patches were removed after 48 hours and the test were read. All these tests were not quantitative.

4.2.2.3 Multiple Puncture Test[40]

This is a modification of the Von Pirquet's and Trumbusti tests. There were multiple punctures instead of two punctures.

4.2.2.4 Heaf Test[40]

This test was introduced by Fredrice Heaf and is called Heaf's Modified Multiple Puncture test. This test was further improved by Rosenthal and Heaf himself. In this test adrenalised tuberculin is sprayed on the skin by using a platinum loop. An instrument called the Heaf's gun is used to puncture the skin with 6 or more needles arranged in a circular metal head 6mm in diameter. The test can be read up to 7 days and is hence called the 'market day test'. Red dots at the test site indicate positivity.

4.2.2.5 Stern Needle Test

The stern needle test is a modification of Heaf Test. It uses detachable metal heads, which can be sterilized in bulk.

4.2.2.6 The Tine Test

This test is done with individually prepared sterilized units. Old Tuberculin is dried on sterilized tine point and used once and discarded. This method cannot quantify allergy.

4.2.2.7 The Dermo Spray Method

This is a modification of the intradermal test technique as designed by Krantz. He designed an apparatus that can inject exactly 0.1 ml of fluid through a capillary under high pressure. The high-speed jet of fluid automatically pierces the skin and produces a pale wheel as in a good Mantoux test. Since the apparatus does not use a needle to pierce the skin, it is enough to sterilize the entire apparatus before each session.

TABLE NO. 1 Latent tuberculosis infection(LTBI) prevalence studies in population using TST

Sr. No	Area & Study Population	Sample size	Type of Tuberculin	Site of Testing	Level of Demarcation	Prevalence of Latent Tuberculosis Infection
1	0-60+ age group of Tumkur district (1960-61)[41]	26062	1 TU RT 23 with Tween 80	Mid Volar surface of left forearm	≥ 10mm	Overall 38.3% (Male 42.8, Female 33.9)
2	0-55+ age group of Magadi Chanapatna,Nelamangala of Bangalore district .Total 119 villages (1961-68). Four times surveyed longitudinal study[42]	1st Survey -50146 2nd Survey- 46625 3rd Survey – 46545 4th Survey – 47207	1 TU RT 23 Tween 80	Right or Left forearm ; alternating the area at each survey but choosing a different site for the test	≥ 10mm	1st Survey –Overall 29.5% 2nd Survey- Overall 30.4% 3rd Survey – Overall 29.3% 4th Survey – Overall 30.4%
3	0-60+ years of Neelamangala subdivision of Bangalore district 1968-1972[42]	29962	1 TU PPD RT 23 with Tween 80	Not available	≥ 12mm	Overall 35.2%

Sr. No	Area & Study Population	Sample size	Type of Tuberculin	Site of Testing	Level of Demarcation	Prevalence of Latent Tuberculosis Infection
4	All ages of Chingelpet district of Tamilnadu in the year 1971[44]	2,63,842	3 TU 0f PPD-S	Mid dorsal aspect of forearm	≥ 12mm	Overall 50%
5	0-55+ age group of Magadi,Chanapatna,Nelamangala taluks of Bangalore district 1977-78[45]	8025	1TU PPD RT 23 with Tween 80	The upper third of the dorsal aspect of the right forearm	≥ 16mm	Overall 65.2%
6	All ages of Srinagar, Bramulla and Anantnag district of Kashmir valley in the year 1978[46]	12832	3TU of PPD-S	Mid dorsal aspect of the forearm	≥ 12mm	Overall 38%

The above table shows the various prevalence studies on latent TB infection in India. The prevalence of latent TB infection range from 29.5% to as high as 65.8%.

TABLE NO. 2 Prevalence of LTBI among contacts of Infective Tuberculosis

Sr. No	Area & Study Population	Sample size	Type of Tuberculin	Level of Demarcation	Prevalence of Latent Tuberculosis Infection
1	Chandigarh , Under 5 contacts of sputum positive patients[47]	140 contacts and 141 contacts of sputum negative patients	1 TU of PPD RT with Tween 23	> 10 mm	46.4% in contacts of sputum positive cases 21.2% in contacts of sputum positive cases
2	Sevagram, Gujrat(2004) Among health Care Workers[48]	720	1 TU of PPD RT with Tween 80	≥ 10 mm	41.3% among all health care workers

This table shows the prevalence of among contacts. The prevalence is between 41.3% and 46.4%.

4.2.3 New alternative test to diagnose LTBI

In the absence of a gold standard to diagnose latent tuberculosis infection, it may be difficult to demonstrate that any test is better than the tuberculin skin test. However, the sensitivity of a potential test may be predicted by correlating its result with the degree of exposure (duration and proximity) to a source patient and the likelihood of acquiring infection from that source. A test would be more sensitive than the tuberculin skin test if it is positive in patients with a high risk of exposure. A more specific test could be independent of vaccination with BCG.

Recently new immune – based blood tests were developed for the diagnosis of latent tuberculosis. These tests measures interferon gamma released in response to stimulation of sensitized T-cell by mycobacterial antigens.[49] The best studied of these antigens are Early Secretory Antigenic Target-6 (ESAT-6) and Culture Filtrate Protein- 10 (CFP-10). These tests are called Interferon Gamma Release Assay (IGRA) tests. There are two commercially available diagnostic tests incorporating specific antigens. They are QuantiFERON TB Gold (Cellestis, Australia) and T-SPOT –TB Assay (Oxford Immunotech, UK).

The sensitivity of IGRA tests were studied in patients with active tuberculosis and contacts of infectious tuberculosis patients. From these studies, in patients with active tuberculosis disease , IGRA tests were found to have a sensitivity of 74-96%, while tuberculin skin test (TST) had a sensitivity of 64-69%.[51,52,53] In contact tracing studies, IGRA tests were found to be as sensitive as TST for detecting latent tuberculosis infection and, in some studies, they correlated better with the degree of exposure.[54, 55, 56]

However, in immunocompromised patients, the data is too scarce to make any definite conclusions regarding the agreement between the two tests. One study of 590 HIV- infected patients showed that QuantiFERON TB Gold (QFT-G) test correlated with known risk factors for latent tuberculosis infection or past history of tuberculosis.[56]

The gold standard proof of latent tuberculosis infection is the eventual development of active tuberculosis disease. This can be observed by conducting a longitudinal study where tested individuals are followed up for development of active tuberculosis disease. Diel et al, in Germany evaluated 601 close contacts of infective tuberculosis patients of whom 278(46.3%) had vaccination with BCG.[57] Tuberculin skin test was positive in 243(43%), while QFT-G was positive in 66(11%) contacts. All contacts were followed for 2 years. Six of the contacts developed active tuberculosis disease during follow-up and all six were QFT-G positive. None of the QFT-G negative individuals developed tuberculosis. This important study concluded that QFT-G is a more accurate predictor of latent tuberculosis infection than tuberculin skin test (TST).

IGRA tests have several operational advantages over tuberculin skin tests. They require only one visit for blood smpling.[57] Automated reading reduces the reader bias in interpretation. There is no booster effect of the test and, therefore, repeated testing (e.g., in health care workers) does not affect results.[57] The test is read in 24 hours. But IGRA tests need some base laboratory and some technical skills.

The main advantage of IGRA tests is their specificity compared to TST.[57] This significantly eliminates false positive results in BCG vaccinated individuals and therefore avoids the cost and toxicity associated with unnecessary treatment. The sensitivity of IGRA tests are found to be similar as TST from studies.[54, 55, 56] In contact tracing studies,

they showed good correlation with the degree of exposure to an index case. [54,55,56] In some studies, sensitivity of IGRA tests was found to be better, particularly among immunocompromised patients.[56] Detection of latent tuberculosis infection in these patients is highly important because of their increased risk of progression to active disease.

The main disadvantage of any IGRA test is their cost. A single assay usually cost around $ 30-40. The cost to the healthcare system may initially increase but the overall cost will decrease, as less latent tuberculosis patients are treated and less visits are required. The question is whether IGRA tests should complement or replace TST. The TST is cheap and sensitive for diagnosing latent tuberculosis infection. However, in BCG vaccinated individuals, its specificity is clearly inferior to that of IGRA tests. The expensive IGRA test may not be affordable by many developing countries. Therefore, it is likely that TST will remain in use in many parts of the world.

In resource- rich countries, IGRA tests are increasingly utilized. In their, guideline, the Centre for Disease Control and Prevention (USA) has suggested replacing TST with QuantiFERON TB Gold [59] On the other hand British guideline recommend using IGRA tests as confirmatory tests in those with positive TST results.[60] A cost analysis study in Germany found that screening of contacts by TST followed by QuantiFERON TB Gold Assay in positive TST reactors was the most cost-effective method for screening for latent tuberculosis infection.[57]

4.2.3.1 QuantiFERON TB GOLD Assay[61]

QuantiFERON TB Gold In- Tube (QFT) is an in vitro diagnostic test using a peptide cocktail simulating ESAT-6 , CFP-10 and TB-7 proteins to stimulate cells in the

heparinised whole blood to release interferon. Detection of interferon- γ (IFN-γ) by Enzyme Linked Immunosorbent Assay (ELISA) is used to identify in vitro responses to these peptide antigens that are associated with Mycobacterium tuberculosis infection.

QFT is an indirect test for M.tuberculosis infection (including disease) and is intended to be used in conjunction with risk assessment, radiography and other medical and diagnostic evaluation.

4.2.3.2 Steps of QuantiFERON TB GOLD Test[61]

a. Patient's blood is collected into blood collection tubes and is mixed by shaking the tubes up and down 10 times to ensure that the entire inner surface of the tube is coated with blood.

   QFT TB Gold uses two types of tube for collection of blood namely

   - Grey cap tube containing nil control
   - Red cap tube containing TB Antigen

b. After collection of the blood into the container it is transferred to an incubator as soon as possible and latest within 16 hours of collection. The sample is not refrigerated or freezed. The tubes are incubated upright at 37℃for 16-24 hours. The incubator does no require carbon dioxide or humidification. After incubation at 37℃for 16-24 hours, the blood collection tubes may be held between 2 to 27° C for up to 3 days prior to centrifugation.

c. After incubation of the tubes at 37° C, harvesting of the plasma is facilitated by centrifuging the tubes for 15 minutes at 2000 to 3000 RCF (g). The gel plug

separates the cells from the plasma. If this does not occur, the tubes are re-centrifuged at a higher speed. This will separate the plasma and the red cells.

d. Plasma samples are loaded directly from blood collection tubes into the QFT-TB Gold ELISA Plates, especially when automated ELISA workstations are used.

e. All plasma samples and regents, except for conjugate 100X Concentrate, are brought to room temperature (22 – 25^{o} C) before use. At least 60 minutes are allowed for equilibrium.

f. The freeze dried "kit standard" is reconstituted with equal volume of deionised or distilled water indicated on the label of the standard vial. It is mixed gently to minimize frothing and to ensure complete solubilisation. Reconstitution of the standard to the stated volume produces a solution with a concentration of 8.0 IU/L. The reconstituted kit standard is used to produce a 1 in 4 dilution series of IFN-γ in Green Dilution (GD).

g. S 1 (Standard I) contains 4 IU/ml , Standard 2 (S2) contains 1 IU/ml , S3 contains 0.25 IU/ml and S4 contains 0 IU/ml (Green Dilution).

h. The freeze dried conjugate 100X Concentrate is reconstituted with 0.3 ml of deionised or distilled water. It is mixed gently to minimize frothing and to ensure complete solubilisation of the conjugate.

i. Prior to assay, plasma is mixed to ensure that IFN-γ in evenly distributed throughout the sample.

j. 50 µL of freshly prepared Working Strength Conjugate is added to the required ELISA wells using a multichannel pipette.

k. 50 µL of test plasma samples is added to appropriate wells using a multichannel

pipette. Finally, 50µL each of the Standards 1 to 4 is added.

l. The conjugate and plasma samples / standards are mixed thoroughly using micro plate shaker for 1 minute.

m. Each plate is covered with a lid and incubated at room temperature (22°C +- 5°C) for 120 +- 5 minutes.

n. During the incubation, one part Wash Buffer 20X conjugate is diluted with 19 parts deionosed or distill water and mixed thoroughly. It is washed well with 400µL of working strength wash buffer for at least 6 cycles. An automated plate washer is used.

o. Residual wash buffer is removed by making the plates face down on an absorbent towel. 100µL of Enzyme Substrate Solution is added to each well and mixed thoroughly using a microplate shaker.

p. Each plate is covered with a lid and incubated at room temperature (22°C +- 5°C) for 30 minutes. Plates are not exposed to direct sunlight during incubation.

q. Following 30 minutes of incubation, 50µL of Enzyme Stopping Solution is added to each well and mixed. Enzyme Stopping Solution is added to the wells in the same order and at approximately the same speed as the substrate.

r. The Optical Density (OD) of each well is measured within 5 minutes of stopping the reaction using a microplate reader fitted with a 450nm filter and with a 620-650nm reference filter. Optical Density values are used to calculate the results.

4.2.3.3 Calculations and Test Interpretation[61]

QFT –TB Gold IT Analysis software is used to analyse raw data and calculate results. This software is available with Cellistis, Australia. If this software is not used, results can be obtained manually.

The mean OD values of the kit standard replicates on each plate are determined. A standard curve is constructed by plotting the log (e) of the mean OD in the y-axis against the log (e) of the IFN-γ concentration of the Standard in IU/ml in the x-axis, omitting the 0 standard from the calculation. The line of best fit for the standard curve is calculated by regression analysis. The standard curve is used to determine the IFN-γ concentration (IU/ml) for each of the test plasma samples, using the OD value of each sample.

TABLE NO. 3 Interpretation of Results when NIL Tube and TB Antigen are used[61]

NIL (IU/ml)	TB Antigen minus Nil (IU/ml)	QuantiFERON TB (IU/ml)	Interpretation
≤ 8.0	< 0.35	Negative	M.Tuberculosis infection NOT likely.
	≥ 0.35 and < 25% of NIL Value	Negative	M.Tuberculosis infection NOT likely.
	≥ 0.35 and ≥ 25% of NIL Value	Positive	M.Tuberculosis infection likely.
> 8.0	Any value	Indeterminate	Results are indeterminate for TB Antigen responsiveness.

The above table shows the interpretation of QFT-G test results when the software is not available.

4.2.3.4 Cautions and Limitations of Using QFT[59]

Certain limitations of QFT-TB Gold are similar to that of the TST, but these limitations have not been studied extensively for QFT-TB Gold. Whereas the sensitivity of QFT-TB Gold for detecting M. tuberculosis infection in persons with untreated culture confirmed TB is approximately 80% in published studies, the QFT sensitivity for particular group of TB patients (eg., young children and immunocompromised patients) has not been studied.

QFT-TB Gold sensitivity for detecting latent tuberculosis infection might be less than that of the TST, although the lack of confirmatory test to detect latent TB infection makes it difficult to assess this. Estimating the sensitivity of any indirect test for latent TB infection, like testing the patients who have tuberculosis disease, might be inaccurate because of differences between these conditions. The ability of QFT to predict risk for progress to tuberculosis disease has not been determined.

QFT, as with the TST, cannot differentiate infection associated with TB disease from latent tuberculosis infection. A diagnosis of latent tuberculosis infection requires that tuberculosis disease be excluded by medical evaluation, which should include checking for suggestive signs and symptoms, a chest radiography, and, when indicated examination of sputum or other clinical sample, the presence of M. tuberculosis.

Similar to any other diagnostic test, the predictive value of QFT results depends on the prevalence of M.tuberculosis infection in the population being tested. Each QFT result and its interpretation should be correct in conjunction with other epidemiological, historic, physical and diagnostic findings.

As with a negative TST result, negative QFT result should not be used alone to exclude M.tuberculosis infection in persons with symptoms or signs suggestive of tuberculosis disease. The presence of symptoms or signs suggestive of TB disease increases the likelihood of M.tuberculosis infection, and this decreases the negative predictive value of QFT or TST test in detecting latent tuberculosis infection. Medical evaluation of such persons should include a history and physical examination, chest radiographs, bacteriological studies, serology for human immunodeficiency virus (HIV), and, when indicated , other tests or studies.

The performance of QFT , in particularly its sensitivity, and its rate of indeterminate results, has not been determined in persons who, because of impaired immune function, are at increased risk for M.tuberculosis infection progressing to tuberculosis disease. Impaired immune function can be caused by HIV infection or acquired by immunodeficiency syndrome (AIDS); current treatment with immunosuppressive drugs including high dose corticosteroids, tumor necrosis factor-α (TNF-α) antagonist and drugs used for managing organ transplantation; selected hematological disorders (e.g., myeloproliferative disorders, leukemias and lymph node specific malignancies); diabetes ; silicosis; and chronic renal failure. Each of these conditions or limitation is known or suspected to decrease responsiveness to the TST, and that might decrease production of IFN-γ in the QFT assay. Consequently, as with a negative TST result, negative QFT alone might not be sufficient to exclude M. tuberculosis infection in these persons.

Published data are relatively limited concerning the use of QFT among persons recently exposed to tuberculosis (i.e., close contacts) and other populations at high risk

for latent tuberculosis infection. No published data document the performance of QFT in children aged < 17 years.

With any of the testing methods, persons who have a negative result can still have latent tuberculosis infection. Those who have negative but who are likely to have latent TB infection and those who are at a greater risk of developing severe illness or poor outcome from TB disease need treatment or close monitoring for disease.

Potential examples include close contacts who aged less than 5 years; or those who are immunocompromised due to HIV infection or those on any immunosuppressive therapy.

QFT has practical limitations that include the need to draw blood and to ensure its receipt in a qualified laboratory in time for testing. The blood must be incubated with the test antigens < 12 hours after collection to keep the lymphocyte viable. After the blood is incubated with antigens for 16-24 hours, plasma must be collected and either properly stored or tested promptly by ELISA. Collecting the required 5 ml blood sample from young children might not be possible or acceptable

4.2.3.5 Agreement Between Tuberculin Skin Test & QuantiFERON TB Gold

Since there is no gold standard test to diagnose latent tuberculosis infection it is difficult to accurately measure the sensitivity or specificity of any test. TST has been used for a long time as a standard test to detect latent tuberculosis infection. So the new test QuantiFERON TB Gold is evaluated based on an agreement i.e concordance with TST in healthy populations with varying risk for latent tuberculosis infections. Some of these studies are summarized in the following table no.4.

TABLE NO.4 Agreement between QuantiFERON and TST in healthy population with varying risk for LTBI

Study, Year Reference	**Country**	**Risk Group**	**Total Participants**	**BCG Vaccinated (in %)**
Brock et al., 2004	Denmark	Contacts of persons with TB	45	0
Pai et al., 2005	India	Health Care Workers	719	71
Kang et al., 2005	Korea	Close & casual contacts of persons with TB	120	73
Porsa et al., 2006	USA	Prisoners	409	-
Ferrara et al., 2006	Italy	Hospitalised adults	286	18
Harada et al., 2006	Japan	Health Care Workers	304	91

Study, Year Reference	Country	Risk Group	Total Participants	BCG Vaccinated (in %)
Dogra et al., 2006	India	Hospitalized children	97	82
Mahomed 2006	South Africa	Healthy adults	358	81
Tsiouris et al., 2006	South Africa	Pediatric contacts	184	73
Lee et al., 2006	Korea	Healthy students	131	100
Nakaoka et al., 2006	Nigeria	Pediatric contacts	179	37
Connell et al., 2006	Australia	Pediatric contacts	75	49

Table 4 shows the various studies comparing the agreement between TST and QFT-G. The agreement between the tests varies from as low as 13% to as high as 94%.

5. MATERIALS AND METHODS

5.1 Study Design

The study was done by a cross sectional design.

5.2 Study Area

The study area comprises of population covered by two Tuberculosis Units (TU) in Vellore district: CHAD TU and DTC TU .CHAD (Community Health and Development) is a 140 bedded hospital run by the Department of Community Health of the Christian Medical College, Vellore. This CHAD TU comprises of 12 peripheral health institutes (PHI) which include CHAD hospital, Narayani hospital and 10 primary health centre (PHCs) run by the government. This TU covers around 6 lakhs population. The other Tuberculosis Unit (TU) is based at District Tuberculosis Centre, Vellore and comprises of 11 PHIs. This includes Christian Medical College and Hospital and 10 other PHCs. This TU covers around 5.5 lakhs populati

5.3 Sample Size

Sample size was calculated using the formula

$N = 4PQ / d^2$

Where N= sample size required

P= prevalence of latent tuberculosis infection among contacts from previous studies

Q = 100-P

d= Relative Precision taken as 20% of Prevalence

Taking prevalence P as 40%[73] the sample size was calculated as **150.**

5.4 Study Population and Sampling Method

Among all the suspected MDR patients in these two TUs a total of 147 were done sputum culture. Out of this 147 patients , 104 found culture positive for M.Tuberculosis. Out of 104 sputum culture positive patients 48 were selected based on the distance of these houses from CHAD within Vellore corporation limits and Kaniyambadi block. 44 of them agreed to participate in the study. From this 44 index cases a total of 154 contacts were identified and subjected to both the tests.

Exclusion Criteria

- Person already diagnosed as having tuberculosis
- Those with signs and symptoms suggestive of tuberculosis
- Those already on chemoprophylaxis for tuberculosis
- Persons known to be immunocompromised
- Children less than 1 year of age

Out of the 38 index cases contacted, 34 agreed to participate in the study. The total number of contacts of these 34 index cases was 154.

5.5 Informed Consent

Information sheet and consent form was written in Tamil and was approved by the Institutional ethics committee. Informed consent was taken from individual subject. For those who are minor consent was taken from the guardian.

5.6 Methodology

2 Tuberculin Unit (2TU) PPD RT 23 with Tween 80 was used for this study. The standard 2TU PPD is manufactured by Statens Serum Institute (SSI), Copenhagen, Denmark and is not generally licensed for use in India. A license to import and use 2TU PPD from SSI, Copenhagen was obtained from the Drugs Controller General of India.

Two medical laboratory technicians were selected to do the testing and reading. Both the technicians underwent a 7 days training on testing and reading tuberculin skin test under the guidance of Dr.A.N.Sashidhara, Senior Field Investigator at National Tuberculosis Institute, Bangalore.

The houses were visited and formal written consent was taken. A structured questionnaire was administered for obtaining socio demographic parameters.

Blood was collected by venepuncture from cubital vein after cleaning the site with normal saline. The samples were collected into the labeled sample collection tube upto the required marks and were kept at ambient temperature.

The mid volar aspect of right forearm was selected for tuberculin skin testing. The site was identified and cleaned with normal saline. 1 ml 2TU PPD was loaded in the tuberculin syringe. The skin was stretched and the 2TU was applied. In case of unsatisfactory test the procedure was repeated further away from the first site.

The tuberculin skin test was read between 48- 72 hours and the results were reported in millimeter. Any unusual reactions such as abscess, ulceration, necrosis, were also recorded.

Children less than 15 years with indurations ≥ 10mm were referred to CHAD hospital for further investigations and management.

5.7 Statistical Analysis

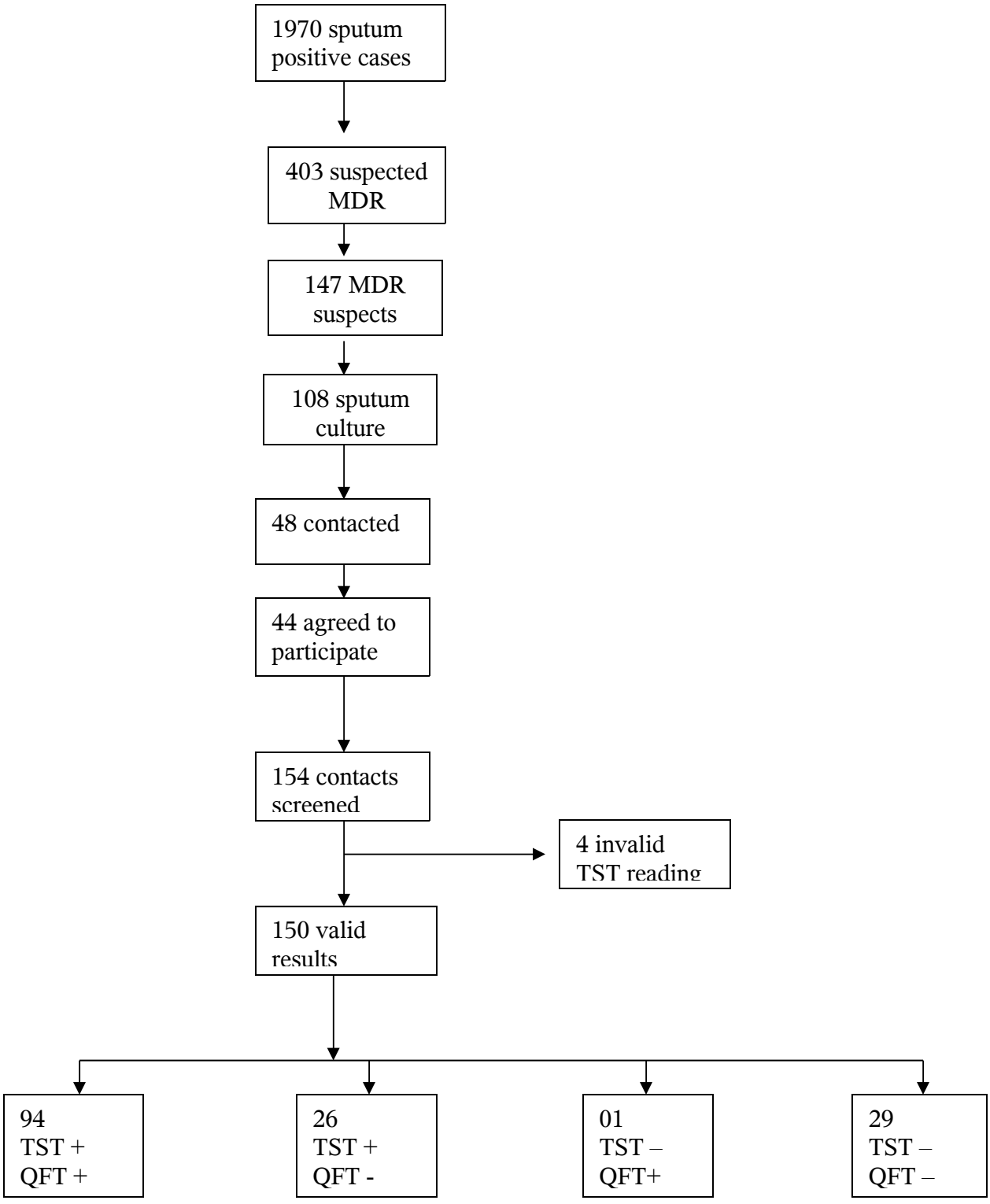
The data were entered and analyzed using EPI INFO 2002 software.

The primary outcome measured was, the prevalence of latent tuberculosis infection using tuberculin skin test with a cut off 10mm and QFT cut off 3.5IU/ml. The data were further stratified into pediatric (<15 years) and adult age group and the prevalence was estimated.

The TST and QFT response was further analyzed in relation to age, sex and, proximity of contact (as measured by sleeping in same or different house as the index case) individually . The association was expressed in terms of chi square and p value .The agreement (both percent agreement and kappa agreement) between TST and QFT was estimated. This agreement was further analyzed in pediatric and adult age group separately.

6. RESULTS

Figure No. 1 Study Flow Diagram



6.1 General Profile

6.1.1 Characteristics of the study population.

Total number of subjects in the study was 150. Out of this 150, 64 were male constituting 42.7% of the population and 86 were female comprising 57.3% of the population.

TABLE NO.5 Age and sex distribution of the study population.

AGE/SEX	MALE	FEMALE	TOTAL
PEDIATRIC	33 (66%)	17 (34%)	50(100%)
ADULT	31 (31%)	69 (69%)	100(100%)
TOTAL	64	86	150

Table 5 shows the age- sex distribution of study population. The total number of subjects in the pediatric age group (< 15 years) is 50 out of which 33(66%) are male and 17(34%) are female. The total number of subjects in the adult age group is 100 out of which 31(31%) are male and 69(69%) are female.

TABLE NO.6 BCG scar status of the study population

AGE GROUP	SCAR PRESENT	SCAR ABSENT	TOTAL
PEDIATRIC	36 (72%)	14 (28%)	50(100%)
ADULT	25 (25%)	75 (75%)	100(100%)
TOTAL	61	89	150

Table 6 shows the distribution of BCG scar in the study population in adult and pediatric age group. Presence of BCG scar is taken as a surrogate for BCG vaccination. Among the pediatric age, 36(72%) have BCG scar and 14(28%) do not have the scar. In the adult population 25(25%) have BCG scar and 75(75%) do not have scar.

6.2 Overall prevalence of LTBI

TABLE NO.7 Prevalence of LTBI by TST

Age Group	TST Positive	TST Negative	TOTAL	CHI SQUARE	P VALUE
Pediatric(<15)	32(64%)	18(36%)	50(100%)	12	**0.001**
Adult	88(88%)	12(12%)	100(100%)		
TOTAL	120(80%)	30(20%)	150(100%)		

Above table shows that the TST positivity is higher in adult age group and this difference is significantly higher in comparison to pediatric age group.

TABLE NO. 8 Prevalence of LTBI by QFT

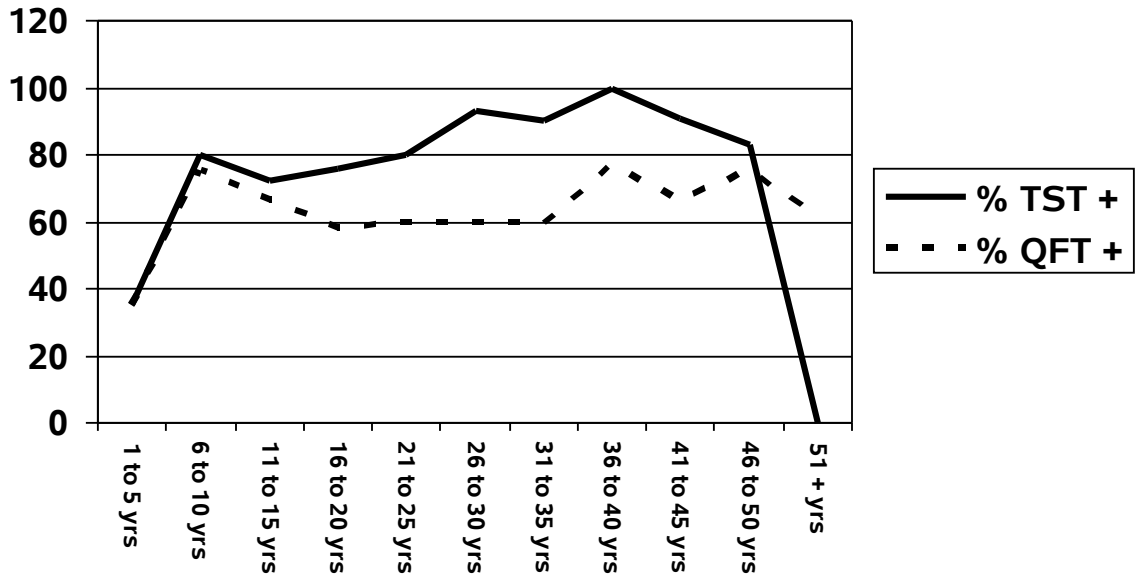
Age Group	QFT POSITIVE	QFT NEGATIVE	TOTAL	CHI SQUARE	P VALUE
Pediatric(<15Yrs)	30(60%)	20(40%)	50(100%)	0.359	**0.545**
Adult	65(65%)	35(35%)	100(100%)		
TOTAL	95(63.3%)	55(36.7%)	150(100%)		

Table no. 8 shows that the QFT positivity in adult and pediatric age group is not significantly different.

TABLE NO.9 Frequency of LTBI by TST and QFT in different age clusters

Table no.9 shows that TST positivity increases with age whereas QFT positivity remains more or less the same which is also seen in figure no. 2 below.

Figure 2 Frequency of Latent TB infection with increasing age



6.2.1 Distribution of TST Indurations in the study

Out of 150 individual the TST indurations varies from 0 mm to 25 mm. The frequency distribution is shown in Figure 3.

Figure No. 3. Tuberculin Reaction

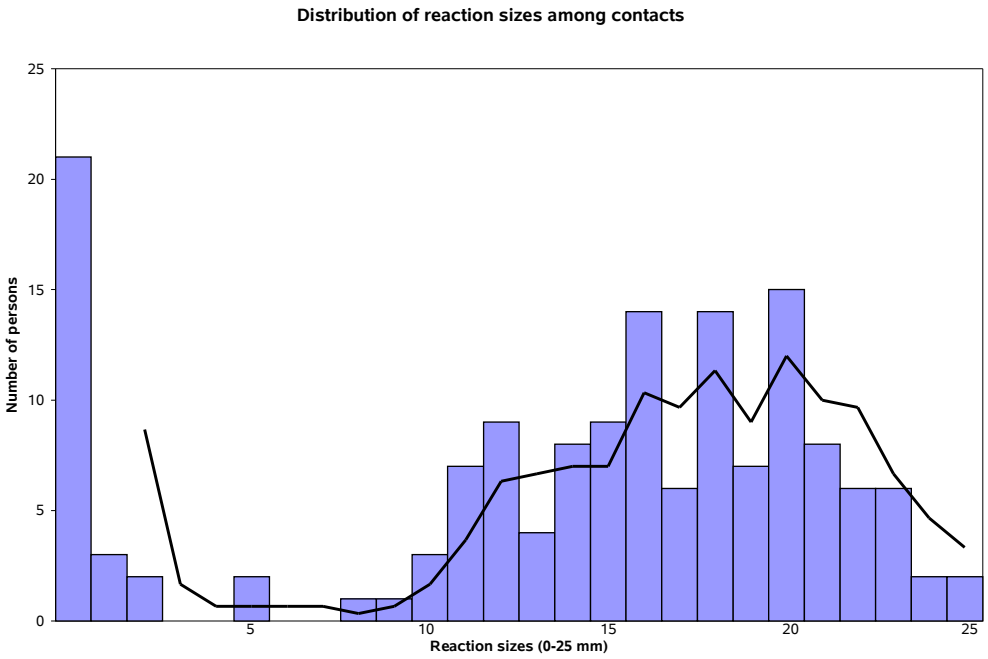


Figure no. 2 shows the frequency distribution of TST indurations in the study sample. There is a bimodal distribution with a fair antimode. Two clear cut modes are visible. One around the 0 mm mark and another around 20 mm mark.

6.3. Prevalence of Latent Tuberculosis Infection in relation to sex

TABLE NO.10 Prevalence of LTBI by Tuberculin Skin Test (TST) in relation to sex

Above table shows that in both age groups there is no significant difference in different sexes in relation to TST positivity.

TABLE NO.11 Prevalence of LTBI by QFT in relation to sex

Table No.8 shows there is no significant difference in different sexes in relation to infection by QFT.

6.4 Prevalence of Latent Tuberculosis Infection in relation to BCG vaccination status

TABLE NO. 12 Prevalence of LTBI by TST in relation to BCG vaccination status

AGE GROUP	BCG SCAR	TST ≥10mm	TST<10mm	TOTAL	CHI SQUARE	P VALUE
Pediatric	Present	24(66.7%)	12(33.3%)	36(100%)	0.66	0.797
	Absent	8(57.1%)	6(42.9%)	14(100%)		
Adult	Present	20(80%)	5(20%)	25(100%)	2.02	0.155
	Absent	68(90.7%)	7(9.3%)	75(100%)		
TOTAL		120(80%)	30(20%)	150(100%)		

Above table shows that there is no significant difference in TST positivity in both ages in relation to BCG vaccination status

TABLE NO.13 Prevalence of LTBI by QFT in relation to BCG vaccination

AGE GROUP	BCG SCAR	QFT POSITIVE	QFT NEGATIVE	TOTAL	CHI SQUARE	P VALUE
Pediatric	Present	22(61.1%)	14(28.8%)	36(100%)	0.66	0.797
	Absent	8(57.1%)	6(42.9%)	14(100%)		
Adult	Present	16(64%)	9(36%)	25(100%)	0.015	0.904
	Absent	49(65.3%)	26(34.7%)	75(100%0		
TOTAL		95(63.7%)	55(36.3%)	150(100%)		

Table 13 shows no difference between BCG vaccinated and unvaccinated in both age groups in having latent tuberculosis infection.

6.5 Prevalence of Latent Tuberculosis Infection in relation to proximity of contact

TABLE NO. 14 Prevalence of LTBI by TST in relation to proximity of contact

AGE GROUP	SLEEPING IN -	TST Positive	TST Negative	TOTAL	CHI SQUARE	P VALUE
Pediatric	Same Room	19(70.4%)	8(29.6%)	27(100%)	1.034	0.309
	Same House	13(56.5)	10(43.5%)	23(100%)		
Adult	Same Room	41(97.6%)	1(2.4%)	429100%)	6.345	**0.012***
	Same House	47(81%)	11(19%)	58(100%)		
TOTAL		120(80%)	30(20%)	150(100%)		

* $p < 0.05$

Table no. 14 shows that there is significant difference in latent tuberculosis infection in adults sharing the same room with index case by TST .

TABLE NO. 15 Prevalence of LTBI

by QFT in relation to proximity of contact

*$p<0.05$ Table no. 15 shows that there is a significance difference in latent tuberculosis infection by QFT positivity in adults sleeping in same room than those sleeping in a different room.

6.6 Prevalence of Latent Tuberculosis Infection in relation to educational status

TABLE NO. 16 Prevalence of LTBI by TST in relation to educational status in adults

EDUCATION	TST POSITIVE	TST NEGATIVE	TOTAL	CHI SQUARE	P VALUE
UPTO PRIMARY	13(100%)	0(0%)	13(100%)	2.038	**0.153**
POST PRIMARY	75(86.2%)	12(13.8%)	87(100%)		
TOTAL	88(88%)	12(12%)	100(100%)		

Table no. 16 shows that although TST positivity is more in lower educational status, this difference is not statistically difference in both educational group.

TABLE NO. 17 Prevalence of LTBI by QFT in relation to educational status in adults

EDUCATION	QFT POSITIVE	QFT NEGATIVE	TOTAL	CHI SQUARE	P VALUE
UPTO PRIMARY	10(76.9%)	3(23.1%)	13(100%)	0.934	**0.334**
POST PRIMARY	55(63.2%)	32(36.8%)	87(100%)		
TOTAL	65(65%)	35(35%)	100(100%)		

Table no. 17 shows that although QFT positivity is more in lower educational status, this difference is not statistically difference in both educational groups as in TST.

6.7 Prevalence of Latent Tuberculosis Infection in relation to occupation

TABLE NO. 18 Prevalence of LTBI by TST in relation to occupation in adults

Table no.18 shows that the LTBI in those at home is more than those working, but this difference is not statistically significant.

TABLE NO. 19 Prevalence of LTBI by QFT in relation to occupation in adults

Table no. 19 shows that the LTBI in those at home is more than those working, but this difference is not statistically significant.

6.8 Agreement between TST and QFT

TABLE NO. 20 Overall Agreements between TST and QFT

	QFT POSITIVE	QFT NEGATIVE	TOTAL
TST POSITIVE	94	26	120
TST NEGATIVE	1	29	30
TOTAL	95	55	150

Table 20 shows that the actual agreement between TST and QFT in detecting latent tuberculosis infection is **82%** and the kappa agreement is **0.57 (95% CI 0.434-0.706).**

TABLE NO. 21 Agreement between TST and QFT in BCG vaccinated individuals

	QFT POSITIVE	QFT NEGATIVE	TOTAL
TST POSITIVE	38	6	44
TST NEGATIVE	0	17	17
TOTAL	38	23	61

Table 21 shows that the actual agreement between TST and QFT in detecting latent tuberculosis infection in all those who are BCG vaccinated is 90% and the kappa agreement is **0.779(95% CI 0.613-0.945).**

TABLE NO.22 Agreement between TST and QFT in BCG unvaccinated individuals

	QFT POSITIVE	QFT NEGATIVE	TOTAL
TST POSITIVE	56	20	76
TST NEGATIVE	1	12	13
TOTAL	57	32	89

Table 22 shows that the actual agreement between TST and QFT in detecting latent tuberculosis infection in all those who are BCG unvaccinated is 76% and the kappa agreement is **0.411(95%CI 0.223-0.599).**

TABLE NO. 23 Agreement between TST and QFT in Pediatric Age Group

	QFT POSITIVE	QFT NEGATIVE	TOTAL
TST POSITIVE	30	2	32
TST NEGATIVE	0	18	18
TOTAL	30	20	50

Table no. 23 shows the actual agreement between TST and QFT in detecting latent tuberculosis infection in pediatric age group is 96% and the kappa agreement is **0.915(95% CI 0.797- 1.033).**

TABLE NO. 24 Agreement between TST and QFT in Adult Age Group

	QFT POSITIVE	QFT NEGATIVE	TOTAL
TST POSITIVE	64	24	88
TST NEGATIVE	1	11	12
TOTAL	65	35	100

Table 2 shows the actual agreement between TST and QFT in detecting latent tuberculosis infection in adult age group is 75% and the kappa agreement is **0.352(95%CI 0.714- 0.533).**

7. DISCUSSION

Tuberculosis is a major public health concern in the world and particularly in India. In countries with high incidence of tuberculosis, the priority is early case detection and treatment. Now there is an increasing concern about the benefits of contact investigation to detect people with latent tuberculosis infection, more so with the spread of MDR tuberculosis. The other issue is, which test to use for detecting latent tuberculosis.

The present study was done in keeping two objectives in mind – first, to detect the prevalence of latent tuberculosis infection in contacts of sputum culture positive tuberculosis among suspected MDR cases and secondly, to find the agreement between TST and QFT in detecting the latent tuberculosis infection.

The study was conducted among the household contacts of 44 culture positive tuberculosis patients. The total number of contacts screened were 150 out of which 64(42.6%) were male and 86(57.3%) were female. The total number of patients in the pediatric age (less than 15 years) were 50(33.3%) out of which 33 were male and 17 were female. In the adult age group, a total of 100 subjects were there, out of which 31 were male and 69 were female.

7.1 Prevalence of Latent TB Infection

Considering the frequency distribution illustrated in figure 3, it can be seen that the entire range of reactions have clustered themselves to form two distinct groups. Both the modes and antimode can be identified. Persons constituting first mode around 0 mm, by and large, are either not reacting at all or reacting very weekly whereas, persons constituting the second mode, around

20mm, are found to be strongly reacting and so they could be logically assumed to compromise of positive reactors specific to tuberculosis infection. From the TST frequency distribution it can be concluded that the TST distribution in the study population more or less follows the same pattern as in general population.[70]

There are various methods to calculate the prevalence of latent tuberculosis infection from this frequency distribution.[71]One method is based on mode-antimode technique. Here, when there is a clear cut antimode, touching the baseline, with two modes, the reactions are identified as those who make up the second mode after the antimode. This method cannot be applied to our study because the antimode touches the baseline twice with a spurt in between.

The other method is the mirror- image technique. In this method, the highest frequency bar is identified from the second mode. The total number of reactors above this is taken (say X). This number is doubled (2X) to make up for both sides of the highest frequency (mirror image). This number (2X) is then added to total number of subjects in the highest frequency (2x + y). This figure is divided by the total number of study subjects (=N) to get the prevalence of latent tuberculosis infection. Using this method the prevalence of latent TB infection is 42%.

According to RNTCP, latent tuberculosis is defined as indurations more than or equal to 10mm.[72] As per the RNTCP definition; out of all 150 subjects, 120 have an induration of ≥ 10 mm i.e. 80%. This figure is high in comparison to other studies done in India.[47, 48] Taking a cut-off of TST at ≥ 5mm the prevalence of latent TB infection is 82% and if we take cut-off of ≥ 15mm the prevalence

drops to 60%.From previous studies done in India as illustrated in table no.1, the prevalence of latent Tb infection in general population varies between 29.3% to 65.0%. In studies among contacts of sputum positive patients in India done by, Singh et al[47] and Pai et al[48] , the prevalence of latent TB varies between 41.3% to 46.4%, as illustrated in table no.2.. This high prevalence (80%) can be explained by the fact that most of the index cases were taken from the "suspect MDR" cases and are likely to have been infective for a longer period. So the contacts have higher chances of getting infection. This is important as we need to know the prevalence of latent infection among contacts of suspected MDR cases as they have potential to develop primary MDR.

7.2 Prevalence of latent TB infection (LTBI) in adult and pediatric age.

Taking a TST cut-off of 10 mm and above, the prevalence of latent infection in the pediatric age group is 64%. In adult age group, the prevalence of latent TB infection, using a cut-off of 10mm and above is 88% as compared to just 65% prevalence obtained by QFT. In pediatric age the LTBI, using QFT is 60% (Table 8). Using TST the LTBI in adult age is more than in pediatric and this difference is statistically significant (Table No. 7).But the difference in LTBI among both the groups are not significantly different using QFT (Table No. 8)

This difference can be explained by the effect of cross reactivity of TST with other environmental mycobacteria, which is much more common in adults than in pediatric age. In case of pediatric age, which is less exposed to environmental mycobacteria than adults, the prevalence of latent TB infection using TST may be

due mainly to M. tuberculosis. This may be the reason why QFT also detects the same proportion of positive reactors in the pediatric age group.

In case of adults, due to probable exposure to environmental mycobacterium, which also renders TST to be falsely positive, we get a large number of TST positives. In the same age group, the QFT positivity is only 65% showing that these positives may be those due to M.tuberculosis thus more or less negating the effect of many environmental mycobacteria. With increasing age, the proportion of people with TST positivity increases while the proportion of adults with QFT positive remains more or less the same(Table No.9; Figure No.2). This may also be due to the effect of environmental mycobacterium on TST.

7.3 Difference in prevalence of LTBI in different groups

In relation to sex (Table No.10 & 11) there is no significant difference between male and female in both adult (in TST p value of 0.394 and in QFT p value of 0.153) and pediatric (in TST p value of 0.584 and in QFT p value of 0.900). This shows that both sexes have equal chances of getting latent infection from the infective case in the household.

In pediatric group, in both BCG vaccinated and unvaccinated individuals there is no significant difference in infection by TST (p value of 0.529) and QFT (p value of 0.797) as evident from table no. 12 & 13. The same is true in the case of adult population (p value with TST is 0.155 and 0.904 with QFT). This indicates that the prevalence of LTBI is not influenced by BCG vaccination.

Comparing the latent tuberculosis infection in adult group in relation to proximity of contact, it is observed that (Table No.14 & 15) contacts sleeping in the same room have a higher chance of getting infection than contacts sleeping in different room(p value with TST is 0.012 and with QFT is 0.030). But in pediatric age group there is no statistically significant difference (p value with TST is 0.309 and with QFT is 0.297) between sleeping in same or different room. From the study it is evident that children are prone to develop LTBI if there is a household contact of infective TB, whether they share the same room or not. This reinforces the stress given to screen household contacts in pediatric group of TB disease.[72]

In case of adults who are educated up to primary class the LTBI is higher than those who are more educated, but this difference is not statistically significant as illustrated in table no. 16 & 17.

Adults who are home are more prone to infection than those who are working outside. This may be due to longer contact in those staying at home(Table 18 &19).

7.4 Comparison between TST and QFT

Many studies have compared the agreement between whole blood interferon gamma (QFT) assay with tuberculin skin test(TST) in low endemic countries.[73] The agreement found in our study (i.e., 84%) is consistent with the findings from majority of the studies in the endemic countries.[62,74,75,76](Table No.4). In one study by Pai et al. among health care workers in India, the

agreement between the two tests was found to be 81.4% which is consistent with our study findings.[48] Another study in 2006 by Dogra et al. among hospitalized children in India have found an agreement of 94% between interferon gamma assay and tuberculin skin test.

With regard to BCG vaccination, previous studies have shown that there is a positive TST/ negative IFN-γ assay discordance in those who are BCG vaccinated. [75, 77] Our study showed that in BCG vaccinated individuals (Table No. 21), the agreement is 90% whereas in BCG non- vaccinated individuals (Table No.22) it is 76%. This discrepancy may be due to misclassification of BCG vaccine status owing to use of scar as a proxy.[32] Recent tuberculin survey in India, involving more than 1 lakh children, have also shown that BCG vaccination does not influence the estimation of annual risk of infection.[3] However, BCG vaccination can have an effect on TST in other populations depending on vaccine strain, timing, frequency and time since vaccination.[3] Another factor is the high prevalence of non-tubercular mycobacterium in India, which tends to cause non-specific sensitivity.[3] Therefore, non tubercular mycobacterium(NTM) infection might have caused false-positive TST results. Because the QFT assays use RDI antigens, the effect of NTM on the test should be limited. However, the two proteins (ESAT-6 and CFP-10) used in QFT have potential to cross react with some of the NTM species.[59] It has been suggested that these proteins may cross react with Mycobacterium leprae.[78] Therefore, more studies are needed to study the effect of non- tubercular mycobacterium and M.leprae on IFN-γ assays as these infections are endemic in India.

The TST is highly sensitive and moderately specific, depending on the population screened with specificity being more unpredictable. Several studies, have shown a positive association between TST response and subsequent risk of active tuberculosis.[8] Randomized trials have shown that treatment of latent tuberculosis infection, diagnosed using TST, reduces the risk of active tuberculosis by 60-90%.[79] TST is simple test with less material cost although it requires skilled testers.

In contrast, the IFN-γ assay(QFT) have higher specificity than TST, limited cross reactivity due to non-tubercular mycobacterium infections, no cross-reactivity due to BCG vaccinations and therefore better correlations with exposure to M. tuberculosis than TST.[18,19,20] Few other advantages of QFT-G includes, only single visit by patient, elimination of subjective errors and no boosting effect.[59] But the most important drawback of QFT-G is the limited evidence of association of QFT is response and subsequent progression to active tuberculosis among contacts of infective TB patients.[57] No trials have demonstrated the efficacy of treatment based on IFN-γ assay results. Another limitation of IFN-γ assay is the need for laboratory infrastructure for the tests and its cost.

8. SUMMARY

A cross sectional study was undertaken among the household contacts of sputum culture positive tuberculosis patients among suspected MDR cases in Vellore, South India. Among a total of 147 suspected MDR cases who underwent sputum culture testing, 104 were found culture positive. Of these 44 household were contacted and 40 household agreed to participate in the study. In these 44 household , 154 household contacts were screened. Valid results were available for 150 individuals only. Of 150 for whom valid results are available, 120(80%), were found to be positive for latent tuberculosis infection by TST and 63.3% by QFT. This showed a high prevalence of latent tuberculosis infection among household contacts of infectious tuberculosis patients who are suspected to have MDR TB.

The prevalence of latent tuberculosis infection among pediatric age group (<15 years) , is 64% and in the adult age group is as high as 88%.This may be due to longer duration of contacts with infective case or due to exposure of adults to environmental mycobacterium. Earlier studies have shown that an early detection and chemoprophylaxis of children with latent tuberculosis infection reduced the incidence of tuberculosis disease and is cost effective. This reinforced the argument that we need to focus on contact tracing, especially among household contacts, to detect more number of latent tuberculosis infections. More studies need to be done to assess the prevalence of LTBI in contacts of special groups like MDR cases.

The study also showed a high agreement between TST and IFN-γ assay in

detecting latent tuberculosis infection. The overall agreement between both the tests is 82% and kappa agreement of 0.571. This high agreement has also been reported by other studies both in endemic and non-endemic countries.

This result has shown that both the tests can be used to detect latent tuberculosis infection. The decision to use any one of them will depend on the setting in which it is used and the resources available. In population, with low tuberculosis infection, where cross-reactivity with other environmental non-tubercular mycobacterium is an issue IFN-γ assay can be used. IFN-γ assay can also be used where serial testing is needed, as in special groups as healthcare workers in contacts with tuberculosis patients. Furthermore, the IFN-γ assay may be helpful in screening population in which low return rates for reading TST is a concern.

In high burden, resource limited countries like India, the TST might still serve a useful purpose. In India, a 15-year follow up of 2,80,000 individuals showed that TST response is significantly associated with development of active tuberculosis.[8] In India, TST is widely used in diagnosing childhood tuberculosis and in epidemiological studies to estimate annul risk of tuberculosis infection.

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9. LIMITATIONS

1. A longer period of training for the readers and testers, as recommended by National Tuberculosis Institute, Bangalore, might have been more valuable.

2. Important covariates like infection with HIV or other non- tubercular mycobacterium infections were not measured.

3. This findings cannot be generalized on all sputum positive cases as the population covered in the study are all treatment failure cases who are MDR suspects.

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11. ANNEXURE

1. Information Sheet: a. Tamil b. English

2. Consent Form: a. Tamil b. English

3. Questionnaire-1

4. Questionnaire – 2

5. Drugs Controller General of India’s License to import and use 2TU -1

6. Drugs Controller General of India’s License to import and use 2TU -1

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ANNEXURE 1 b INFORMATION SHEET (ENGLISH)

Prevalence of latent tuberculosis infection among contacts of sputum culture positive tuberculosis patients in and around Vellore, using whole blood interferon gamma and tuberculin skin test.

Information Sheet

You are invited to participate in the above mentioned study. This is a research project done towards the completion of a MD thesis. The principal investigator of the study is Dr. Satyajit Pattnaik, of the department of Community Medicine, Christian Medical College, Vellore. The duration of your participation in the study will be roughly about 1 month.

In several studies it has been shown that people who are household or neighborhood contacts of patients whose sputum has grown the tuberculosis bacteria, are at high risk for tuberculosis disease. At present the Mantoux test (tuberculosis skin test) is the test used for screening for tuberculosis infection among contacts. Another new test, Quantiferon gold – a blood test, is also available for this purpose.

If you/your child is/are a household or neighborhood contact of a patient whose sputum has grown tuberculosis bacteria, then you/your child will be eligible to participate in the study. As a part of the study, some basic questions will be asked about your personal information and contact history with the tuberculosis patient. You will have to undergo a skin test which will detect whether you have tuberculosis infection or not. On the day of the study an injection will be placed on the left forearm. The study team will return after 3 days to read the skin test. The reports will be notified to you. You may have to give 3ml blood for another test subsequent to the result of first test. In case you are detected to have tuberculosis infection, you will be offered further evaluation and treatment at CHAD hospital as per the government tuberculosis protocol. Both these tests have been proven to be safe and except mild reaction for the tuberculosis skin test, there are no foreseeable risks. The results of your/your child's test will be kept confidential and will not be revealed to anybody. The participation in this study is purely voluntary and you may withdraw from the study at any point without any notification.

If you have any further doubts or clarifications you may contact the principal investigator at the following address:

Dr. Satyajit Pattnaik
Community Health Department,
Christian Medical College, Bagayam
Phone – 0416-2284207
Mobile – 9944876043

ANNEXURE 2 a CONSENT FORM (TAMIL)

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Ýó£Œ„CJ¡ ªðò˜ -«õÖK½‹ Üî¡ ²Ÿø^F½‹, êOJ™ è£ê«ï£Œ A¼I è™êK™ õ÷˜„Cò¬ì‰î «ï£òOèÀì¡ i†®«ô£ Ü,è‹ð,è^F«ô£ ªî£ì˜¹¬ìòõ˜èO¬ì«ò è£ê«ï£Œ Þ¼Šð¬î, è‡ìPò,

è£ê«ïŒ «îŒ™ ðK«ê£î¬ù ñŸÁ‹ °õ£¡®çªðó£¡ «è£œ† âùŠð´‹ ðK«ê£î¬ù ÜAòõŸP¡ Ýó£Œ„C.

ðƒ°ªðÁðõ˜ ªðò˜ _____
õò¶ _____ Hø‰îï£œ _____

(1) Þ‰î îèõ™ ð®õˆ¬î ð®ˆ¶ ÞF™ Þ¼,°‹ Mõóƒè¬÷Š ¹K‰¶ªè£‡«ì¡. âù,° «èœMèœ «è†,è õ£ŒŠðO,èŠð†ì¶.
(2) Þ‰î Ýó£Œ„CJ™, ï£¡/â¡ °ö‰¬î ðƒ° ªðÁõ¶ ²òM¼Šðˆ F¡ «ðK«ô«ò â¡Á ÜPA«ø¡. è£óí‹ â¶¾‹ Ãø£ñ™ âŠªð£¶ ªõ‡´ñ£ù£½‹ MôA,ªè£œ÷ âù,° àP¬ñ à‡´ â¡Á ÜPA«ø¡.
(3) â¡/â¡ °ö‰¬îJì‹ «êèK,èŠð´‹ Mõóƒèœ Ýó£Œ„Cò£÷˜èœ âŠ«ð£¶«õ‡´ñ£ù£½‹ àð«ò£èŠð´ˆF,ªè£œ÷ ï£¡ ê‹ñF,A«ø¡. ï£¡ Ýó£Œ„CJL¼‰¶ MôA, ªè£‡ì£½‹ Üõ˜èœ Ü ‰î Mõóƒè¬÷Š ðò¡ð´ˆF,ªè£œ÷ ï£¡ ê‹ñF,A«ø¡. Ýù£™ â¡/â¡ °ö‰¬îJ¡ îQïð˜ Ü¬ìò£÷ƒèœ ÞóèCòñ£è ¬õˆ¶,ªè£œ÷Šð´‹ â¡Á ÜPA«ø¡.
(4) Þ‰î Ýó£Œ„CJ™ ªîP»‹ Mõóƒè O¡ M…ë£ùŠÌ˜õñ£ù àð«ò£èˆFŸ° ï£¡ ê‹ñî‹ ÜO,A«ø¡.
(5) «ñŸ°PŠH†ì Ýó£Œ„CJ™ ï£¡/â¡ °ö‰¬î ðƒ°ªðø ï£¡ ê‹ñF,A«ø¡.

ªðŸøõ˜/è£Šð£÷˜/ê†ìÌ˜õñ£ù HóFGFJ¡ ¬èªò£Šð‹ _____

«îF _____--

ªðò˜ _____

Ýó£Œ„Cò£÷˜ ¬èªò£Šð‹ _____

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ê£†„CJ¡ ¬èªò£Šð‹ _____

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ªðò˜ _____

ANNEXURE 2 b CONSENT FORM (ENGLISH)

Study Title: Prevalence of latent tuberculosis infection among contacts of sputum culture positive tuberculosis patients in and around Vellore, using whole blood interferon gamma and tuberculin skin test.

Subject's Initials: _____ Subject's Name: _____
Date of Birth / Age:_____

(i) I confirm that I have read and understood the information sheet dated _____ for the above study and have had the opportunity to ask questions. [ ]
(ii) I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. [ ]
(iii) I understand that the principal investigator, others working on the investigator's behalf, the Ethics Committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the trial. I agree to this access. However, I understand that my identity will not be revealed in any information released to third parties or published. [ ]
(iv) I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s) [ ]
(v) I agree to take part in the above study. [ ]

Signature (or Thumb impression) of the Subject/Legally Acceptable Representative:_____
Date: _____/_____/_____
Signatory's Name: _____

Signature of the Investigator: _____
Date: _____/_____/_____
Study Investigator's Name: _____

Signature of the Witness: _____
Date:_____/_____/_____
Name of the Witness: _____

ANNEXURE 3 QUESTIONAIRE 1

TST – QFT STUDY 2009

CHAD, CMC VELLORE, TAMIL NADU. INDIA

Index Case No.

Contact Case No.

Name of Contact Age: Sex:

Relationship with Index Case:

Education:

Occupation:

History of Tuberculosis:

Any symptoms of Tuberculosis (List all symptoms) :

Sleeping (in relation with Index Case): Same room / Same House but different room / Different House

Average number of hours spent with Index Case (per Day) :

ANNEXURE 4 QUESTIONAIRE 2

TST – QFT STUDY 2009

CHAD, CMC VELLORE, TAMIL NADU. INDIA

Index Case No.

Contact Case No.

Name of Contact Age: Sex:

Relationship with Index Case:

Education:

Occupation:

History of Tuberculosis:

Any symptoms of Tuberculosis (List all symptoms) :

Sleeping (in relation with Index Case): Same room / Same House but different room / Different House

Average number of hours spent with Index Case (per Day) :

No. 3(D/R)/08DC Pt. MI

From:

The Drugs Controller General (India),
Directorate General of Health Services,

FDA Bhawan, Kotla Road, New Delhi.
Dated : 21 JUL 2008

To

Community Health Department
Christian Medical College,
Vellore – 632 002
(India)

Dear Sir,

With reference to your letter No. Nil Dated 24/04/2008 received on 26/6/2008, I am to forward herewith Import Licence No. T-280/08D for the drug / drugs mentioned in your application(s).

Kindly acknowledge receipt of this letter and its enclosures.

Yours faithfully,

Drugs Controller General (India)

Copy together with a copy of licence No. T-280/08D
Forwarded for information to:-

1. The Asstt. Drugs Controller, India, New Customs House, Mumbai
2. The Asstt. Drugs Controller, India, Custom House, Calcutta
3. The Asstt. Drugs Controller, India, custom House, Chennai
4. The Asstt. Drugs Controller, India, IGI Airport, Air Cargo Unit, New Delhi

THE DRUGS AND COSMETICS RULES, 1945
(SEE RULE – 33)

FORM-11

LICENCE TO IMPORT DRUGS FOR THE PURPOSE OF EXAMINATION, TEST OR ANALYSIS

Number of Licence No. T-280/08D

Community Health Department, Christian Medical College, Vellore – 632 002 (India) hereby licenced to, import from Statens Serum Institute, Copenhagen, Denmark the drugs specified below for purposes of examination, test or analysis at your Institute or in such other places as the licensing authority may from time to time authorize.

(1) The licence is subject to the conditions prescribed in the Rules under the Drugs and Cosmetics Act, 1940.

(2) This licence shall unless previously suspended or revoked, be in force for a period of one year from date specified below:

S. No.	NAME OF DRUGS	QUANTITIES WHICH MAY BE IMPORTED
1.	Tuberculin PPD RT 23 SSI 2 T.U. / 0.1 ml	10 vials

*Not for Commercial Purpose.

NOT TO BE UTILIZED IN ANY TESTS AND TRIALS INVOLVING HUMAN VOLUNTEERS/SUBJECTS

ITEM (ONE) ONLY

Place : New Delhi
Date : 21 JUL 2008

LICENSING AUTHORITY

Drugs Controller (India)
Directorate General of Health Services

Gov 2218
27/2/09

No. 3(D/R)/08 DC Pt. MI

From
Drugs Controller General (India)
Directorate General of Health Services

FDA Bhawan, Kotla Road,
New Delhi, Dated 1 APR 2009

To

M/s. Christian Medical College,
Community Health Department,
Vellore- 632 002

Sub: **Amendment in Test Licence No. T- 280/08D-reg.**

Ref: **Your Letter No. Nil dated 05/02/2009.**

Sir,

As requested vide your above quoted letter, the Test Licence No. 280/08D dated 21/07/2008 is hereby **amended to remove** the clause *"Not to be utilized in any tests/trials involving, human subjects/volunteers"*, as the drug Tuberculin PPD RT 23 SSI 2 T.U. /0.1 ml (10 vials) being imported from Denmark is to be used in Bioequivalence study.

Yours faithfully,

(DR. SURINDER SINGH)
Drugs Controller General (India)

डा. सुरिन्दर सिंह
Dr. SURINDER SINGH
औषधि नियंत्रक (भारत)/Drugs Controller (India)
स्वास्थ्य सेवा महानिदेशालय
Dte. General of Health Services
निर्माण भवन/Nirman Bhawan
नई दिल्ली/New Delhi